UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte BRYAN S. WANG, and CARL O. PABO

Appeal No. 2006-3085
Application No. 09/636,243

ON BRIEF

Before ADAMS, MILLS, and LEBOVITZ, Administrative Patent Judges.

LEBOVITZ, Administrative Patent Judge.

DECISION ON APPEAL

This appeal involves claims to zinc finger complexes of two or more fusion proteins, each fusion protein comprising a zinc finger joined to a peptide linker. The Examiner has rejected the claims as lacking written description, indefinite, and anticipated. We have jurisdiction under 35 U.S.C. § 6(b). We reverse the rejections, but set forth a new ground of rejection in which we find all claims obvious in view of prior art.

Background

Zinc finger proteins are DNA-binding proteins. Specification, page 1, lines 11-12. A single finger domain is about 30 amino acids long and recognizes a specific sequence

of nucleotides. Id., page 1, lines 15-16 and 23-30; page 7, lines 18-20. Protein engineering has been utilized to generate zinc fingers with novel DNA sequence specificities. Id., page 2, line 3-page 3, line 16. Because of their DNA-binding properties, zinc finger proteins have been adapted to a variety of different applications, including for sequence detection of target nucleic acid in a sample (id., page 27, lines 6-7) and to regulate gene expression (id., page 27, lines 27-32).

The instant application describes zinc finger proteins that have been appended with peptide sequences which are able to interact and bind to each other. The peptide sequences facilitate the formation of zinc finger protein complexes in which the zinc finger proteins are held together by interactions between the peptides and other contacts the peptides make. Id., page 11, lines 26-34.

Discussion

Claim construction

Claims 5, 6, and 20 are appealed. These are the only pending claims in the application. Claim 5 is the only independent claim and it reads as follows:

> 5. A zinc finger complex, comprising two or more fusion proteins, each fusion protein comprising a zinc finger protein and a peptide linker, wherein the fusion proteins are joined to each other by specific binding of the peptide linkers, and wherein the peptide linkers are non-naturally occurring peptides.

The claim is drawn to a complex which comprises at least two fusion proteins which are "joined to each other by specific binding of the peptide linkers." To understand this structure, we must first look at the construction of the fusion protein.

A fusion protein, according to the claim, comprises a zinc finger protein and a peptide linker. A "zinc finger protein" is defined in the Specification to be a protein which "binds DNA in a sequence-specific manner." Specification, page 7, lines 18-20. A zinc finger protein can contain from one to thirty-seven individual fingers, each finger of which binds to a defined subsite within the target DNA site. Id., page 14, lines 12-17. A zinc finger protein can be engineered to vary the order and nucleotide sequence specificity of its finger components. Id., page 14, line 22-page 15, line 15; page 7, lines 22-27.

A "peptide linker" is not expressly defined, but in the context of the Specification would be understood by the skilled artisan. The "Summary of the Claimed Invention" refers to both "dimerizing peptides" and "peptide linkers." Id., page 3, lines 22-25 and 26-31, respectively.

The dimerizing peptides "mediate association" of the zinc fingers to which they are attached. Id., page 11, lines 8-14. According to the Specification, a phage display method can be utilized to select the dimerizing peptides. Id., page 11, lines 15-22. In the description of this method, it is stated that "phage display is used for selection of linkers." (Emphasis added.) Id., page 19, line 22. "The method involves the generation of diverse libraries of peptides, typically linked to the same zinc finger protein, followed by affinity selection for phage bearing peptides with dimerizing activity." (Emphasis added.) Id., page 19, lines 22-25. In this context, the skilled artisan would recognize that the peptides which are "linked" to the zinc finger proteins are the "peptide linkers" recited in the claims. When the peptide linkers are selected for their ability to associate with each other (i.e., dimerize), they can also be referred to as "dimerizing peptides."

The limitation in claim 5 that the "fusion proteins are joined to each other by specific binding of the peptide linkers" requires that the peptide linkers attach to each other (i.e., "bind"), the same activity defined for the dimerizing peptides. As the "peptide linkers" have the same activity as the dimerizing peptides and also the same physical association with zinc fingers, we consider the two to be equivalent for the purposes of claim 5.

On page 15 of the Specification, the phrase "peptide linkers" is used to describe peptides which covalently join portions of the zinc fingers together. Not until the peptide linkers are selected for dimerizing activity would they be characterized as dimerizing peptides or the peptide linkers of claim 5 which join the fusion proteins together by "specific binding." Thus, it is clear in the context of the Specification that the claimed peptide linkers are peptides which have been selected for their ability to associate the fusion proteins by specific binding (i.e., homo- or hetero-dimerization as described in the Specification on page 11, lines 8-14).

The peptide linkers are characterized by the claim as "non-naturally occurring peptides." The term "non-naturally occurring" is defined in the Specification to refer to "objects and sequences not found in nature." Id., page 8, lines 3-4. Typical and preferred non-naturally occurring sequences are described. The examples in the Specification describe the selection of peptides with dimerizing activity. See, e.g., id., page 19, lines 24-25. The dimerizing peptides are present in fusion proteins where they are linked to zinc finger proteins. Id., page 19, lines 32-34; page 20, lines 1-7; page 28, lines 15-18. In these cases, the term "peptide" is being used as shorthand for the amino acid sequence which is contained within the fusion protein. It is the amino acid

sequence of the dimerizing peptide which possesses the claimed "specific binding" activity enabling them to dimerize the fusion proteins. Accordingly, we interpret the phrase "non-naturally occurring peptides" to mean that the peptide linker sequence (i.e., the dimerizing "peptide linker") is not found in nature.

Consistent with this construction, the Specification states that the selection of "peptides" having "novel" dimerization motifs makes it less likely that they will react "with natural dimerization interfaces presented by proteins in the cell." Id., page 38, lines 30-34. That is, a naturally occurring sequence (i.e., "peptide linker" of claim 5) within the fusion protein is disfavored because it would be more apt to specifically bind to its natural dimerizing partner within a normal cellular protein than a sequence which is artificial ("non-naturally occurring"). In this context, both the peptide and the "natural dimerization interface" are amino acid sequences.

The fusion proteins are joined "by specific binding of the peptide linkers." The Specification does not define "specific binding" in the context of the peptide linkers, but it does state that the peptides "mediate association" of the fusion proteins and that the "proteins ... bind to each other via the dimerizing peptides." Id., page 11, lines 8-14 and page 13, lines 5-6, respectively. The association/binding is characterized as involving "supporting interactions [that] can include contacts between the peptides and/or contacts between a peptide and another region of the protein." Id., page 11, lines 30-34. It is not a covalent interaction involving covalent bonds.

In sum, the claim is drawn to a complex of at least two fusion proteins, where each protein comprises a zinger finger which is fused to a peptide sequence. The

peptide sequence is not found in nature (i.e., non-naturally occurring). Contacts between the peptide sequences in each fusion protein hold the complex together.

Rejection under § 112, first paragraph for lack of written description

Claims 5, 6, and 20 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the Specification. The Examiner stated that the "as-filed Specification does not describe a claimed zinc finger complex comprising two or more fusion proteins linked by peptide linkers that are non-naturally occurring peptides." Answer, page 4.

"To fulfill the written description requirement, the patent Specification 'must clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed.' In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989). An applicant complies with the written description requirement 'by describing the invention, with all its claimed limitations.' Lockwood v. American Airlines, Inc., 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (1997)." Gentry Gallery v. The Berkline Corp., 45 USPQ2d 1498, 1502-1503 (Fed. Cir. 1998).

Claim 5, as originally filed, reads as follows:

> 5. A zinc finger complex, comprising a first fusion protein comprising a first zinc finger protein and a first peptide linker and a second fusion protein comprising a second zinc finger protein and a second peptide linker, wherein the first and second fusion proteins are complexed by specific binding of the first and second peptide linkers, and wherein the first and second peptide linkers are nonnaturally occurring peptides.

Comparing amended claim 5, which is now on appeal, to original claim 5, we find almost all its key claimed limitations, including that the fusion proteins each comprising

a "zinc finger protein" and "peptide linker", that the fusion proteins are joined ("complexed" in the original) by "specific binding", and that the peptide linkers are "non-naturally occurring peptides." The only substantial disparity is that amended claim 5 recites that the complex can comprise "two or more fusion protein," while the original did not contain this express limitation. The Examiner argued that the amended claim lacked support in the disclosure, stating that the disclosure "does not describe two linkers each linked to each ZIF [zinc finger protein] wherein the linkers specifically binds to each other to form a fusion of two zinc fingers as schematically depicted by appellants at page 9 of the 8/8/03 Brief." Answer, page 7. As we understand it, the Examiner's position appears to be that there is no written description for complexes which contain more than two zinc finger fusion proteins held together by specifically binding peptide linkers.

To resolve this issue, we turn to the written description. First, we address whether there is support in the Specification for the claimed subject matter of two fusion proteins held together by specific binding between peptide linkers. We discuss this issue in more detail because the Examiner appears to question whether this embodiment was described in the Specification.

As we have construed the claim, the recited "peptide linker" having "specific binding" activity is the same element referred to in the Specification as a "dimerizing peptide." The skilled worker would have gleaned this concept upon reading the Specification in its entirety. For example, in the "Detailed Description," pages 11-14 are devoted to a description of how peptides are selected from peptide libraries, particularly

random peptide libraries, to identify sequences capable of associating two molecules together (i.e., dimerizing).

The relationship between the peptide linkers utilized in the phage library and the dimerization peptides is also shown in Specification Fig. 3. Fig. 3A is a drawing of a DNA construct containing zinc fingers fused to random peptides ("peptide library"). Fig. 3B shows a dimer of fusion protein products of the DNA construct bound to target DNA. It would be evident that the peptide ("peptide library") linked to ZiF12 (a zinc finger protein) depicted in Fig. 3A, after it has been selected for the dimerizing activity, is equivalent to the "dimerizing peptides" of Fig. 3B

The examples in the Specification also provide support for the written description of the claimed subject matter. On page 32 ("Results"), it is stated that "[t]o select dimerization motifs, we attached random peptides to a DNA-binding domain and selected those fusion proteins that could bind more stably to a symmetric DNA site." The random 15- and 30-amino acid peptides were "expressed at the amino terminus of the first two zinc fingers of Zif268." Id. The zinc finger component mediated binding to target DNA. The binding of monomers – a single fusion protein containing a peptide and zinc finger – and dimers – two of the fusion proteins associated together by the peptide linker – to DNA were characterized. Id., Table 1; page 33, lines 25-page 34, line 7. These experiments clearly demonstrate Appellants' possession of the claimed zinc finger complexes containing two zinger fingers joined to each other by binding between the peptide linkers. We do not find these examples to be incongruent with the Specification's general description as the Examiner contends. Answer, page 9.

We also find support in the Specification for the limitation that the complex contains "two or more" fusion proteins. As pointed out by Appellants (Brief, page 9), it is stated on page 12 of the Specification that dimerizing peptides "are useful for mediating multimerization of zinc finger proteins." A multimer is defined as a "protein made up of more than one peptide chain,"[1] and would include dimers of two fusion proteins (Fig. 3) and trimers of three (Brief, page 9). Although the only examples are of dimers, the Specification refers to these as a "typical application" of the technology, but does not exclude other embodiments. Specification, page 12, lines 21-22. On page 15 of the Specification, it is expressly stated that "[t]wo or more zinc finger proteins can be linked either covalently or by dimerization." Id., page 15, lines 4-5. It is our view this provides explicit support for the claim language (added by amendment on December 27, 2002) "two or more fusion proteins."

For the foregoing reasons, it is our view that the Specification provides an adequate written description of the claimed subject matter. This rejection is reversed.

Rejection under § 112, second paragraph

Claims 5 and 20 stand rejected under 35 U.S.C. § 112, second paragraph, as failing to point out and distinctly claim "the subject matter which the applicant regards as his invention." The Examiner stated that "[i]t is not clear within the claimed context as to what constitutes a [sic] non-naturally-occurring peptide linkers." Answer, page 5. Appellants challenged the rejection, arguing that "non-naturally occurring" is clearly

[1] Zaid et al., Glossary of biotechnology and genetic engineering, FAO Research and Technology Paper, 158 (1999).

defined in the Specification to include only those sequences not found in nature. Brief, page 11.

A specification must conclude with claims "particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention." 35 U.S.C. § 112, second paragraph (2000). The purpose of §112, second paragraph, is to "reasonably apprise those skilled in the art of the scope of the invention." Miles Labs., Inc. v. Shandon, Inc., 997 F.2d 870, 875, 27 USPQ2d 1123, 1126 (Fed. Cir. 1993).

We agree with Appellants that "non-naturally occurring," which is recited in claim 5, would "reasonably apprise" the skilled artisan of the scope of the claimed subject matter. In particular, page 8, lines 3-4, of the Specification states that "non-naturally[ ] occurring is used to describe objects and sequences not found in nature." We find no merit in the Examiner's arguments characterizing the description of non-naturally occurring in the Specification as inconsistent with the "conventional wisdom of the art," and confusing in its reference to protein folding. Answer, page 12, paragraph 2. The statements referred to by the Examiner described "preferred," "typical," and "some" embodiments, which are examples of non-naturally occurring sequences.

For the reasons set forth above, we reverse this rejection.

Rejection under § 102(b)

Claims 5 and 20 stand rejected under 35 U.S.C. § 102(b) as anticipated by Pomerantz.[2]

Pomerantz describes the "design of a dimeric zinc finger protein, ZFGD1, containing zinc fingers 1 and 2 from Zif 268 and a portion of the dimerization domain of GAL4." Pomerantz, Abstract. The GAL4 dimerization domain is utilized to associate two chimeric proteins, each containing a zinc finger fused to the GAL4 domain, i.e., a zinc finger-GAL4 fusion. Id., page 966, column 2; Fig. 1. GAL4 is a naturally-occurring protein that contains a coiled-coil dimerization motif that mediates "protein-protein interaction." Id. Binding studies of the fusion protein established that ZFGD1 binds to DNA as a dimer. Id., page 967, column 2.

The Examiner argued that Pomerantz's disclosure of the zinger finger-GAL4 fusion protein anticipates claims 5 and 20.

> Pomerantz recites that a portion of Gal4 is used as dimerizing linker. This portion is shown at page 967, col. 1 under the heading section RESULTS i.e., the portion that binds to the 13-residue DNA substite [sic]. Read in the light of the specification definition of a non-naturally occurring peptide linkers e.g., less than 50% (amino acid) with natural sequences the GAL4 (41-100 residues) is less than 50% of the naturally occurring sequence of Gal.

Answer, paragraph spanning pages 13-14, emphasis removed.

Appellants contended that "[t]he claims on appeal clearly require linking of two or more proteins via non-naturally occurring peptides. In contrast, the dimerizing linker used by Pomerantz, namely amino acids 41 to 100 of GAL4, is clearly a naturally-occurring peptide sequence, inasmuch as it is part of the naturally occurring

[2] Pomerantz et al., (Pomerantz), Biochemistry, 37(4):965-70 (1998).

GAL4 protein." Supplemental Brief, page 8, paragraph 2. We agree with Appellants' position.

Anticipation requires a showing that each element of the claim is identifiable in a single reference. Perricone v. Medicis Pharm. Corp., 432 F.3d 1368, 1375, 77 USPQ2d 1321, 1325 (Fed. Cir. 2005). The GAL4 domain utilized in Pomerantz's fusion protein is a naturally-occurring sequence obtained from the naturally-occurring GAL4 protein. Having interpreted the claims to require that the peptide linker with specific binding ("dimerizing") activity is a non-naturally occurring sequence, we are compelled to conclude that Pomerantz does not teach each and every element of the claimed subject matter. Consequently, we find that the Examiner has failed to establish adequate evidence of prima facie anticipation. This rejection is reversed.

New Grounds of Rejection

Under the provisions of 37 CFR § 41.50(b), we enter the following new grounds of rejection.

Claims 5, 6, and 20 are rejected under 35 U.S.C. § 103(a) as unpatentable over Pomerantz in view of Krylov.[3]

The Pomerantz publication has been described above for its disclosure of a zinc finger fused to the naturally occurring dimerization domain extracted from the GAL4 protein. Pomerantz's fusion protein differs from the fusion protein contained in the zinc finger complex of claim 5 by having a naturally occurring dimerization domain, instead

[3] Krylov et al. (Krylov), The EMBO Journal, 13(12):2849-61 (1994).

of the non-naturally occurring sequence ("peptide linker") required by the claim. However, in addition to its disclosure of a zinc finger-GAL4 fusion protein, Pomerantz suggests that other dimerizing domains may be appended to the zinc finger.

> The dimerization interface also provides opportunities for further elaboration and optimization. As demonstrated by many studies, the coiled-coil interaction motif offers the potential to modify the dimerization domain to increase dimerization affinity or to specifically promote heterodimer formation (see refs 19 and 20 for examples).
>
> ....
>
> Dimer contacts of modest affinity may allow self-assembly at the appropriate binding site and thereby reduce the risks of nonspecific (kinetic) trapping that may occur with large covalently linked sets of fingers. Cooperative binding, by giving a more dramatic concentration dependence, may also allow more precise on/off switching in targeted gene regulation.

Pomerantz, page 970, column 1.

A prima facie case of obviousness requires evidence that the prior art disclosed or suggested all of the elements of the claimed invention, and that those skilled in the art would have been motivated to combine those elements with a reasonable expectation of success. See In re Wilson, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970); In re Vaeck, 947 F.2d 488, 493, 20 USPQ2d 1438, 1443 (Fed. Cir. 1991).

Here, Pomerantz does not teach that the zinger finger protein is attached to a non-naturally occurring dimerization domain as required by claim 5, but suggests that such domains be used "to increase dimerization affinity," "to specifically promote heterodimer formation," and "allow more precise on/off switching in targeted gene regulation." Pomerantz, page 970, column 1. This provides the motivation which would have led one of ordinary skill in the art to have replaced the GAL4 dimerization domain with non-naturally occurring sequences having the requisite dimerizing activity.

Pomerantz points the skilled artisan directly to prior art publications that teach modified dimerization domains. Such domains are non-naturally occurring and "join each other by specific binding," meeting the requirements of the claimed "peptide linkers." See claim 5. In particular, reference 19 (hereinafter "Krylov"), cited by Pomerantz for its studies of the coiled-coil interaction motif, describes "protein design rules that can be used to modify leucine zipper-containing proteins to possess novel dimerization properties." Krylov, page 2850, column 1. "33 different leucine zipper proteins containing 27 different systematic combinations of amino acids" were produced. Id., page 2856, column 2 ("Discussion"). See also Fig. 1B for a list of exemplary "mutant proteins." Id., page 2850, column 2. The mutant proteins were mixed together under conditions which facilitated dimer formation. By measuring the stability of the dimers formed (id., page 2852-53, "Thermodynamic stability"), Krylov was able to demonstrate that certain modified dimers had increased stability and specificity as compared to the unmodified form. ("Novel heterologous interactions regulate dimerization specificity. ... In the second mixing experiment, the stability of the heterodimer is calculated to be greater than the average of the two homodimer stabilities, thus favoring the formation of heterodimers." Id., page 2856, columns 1-2.) Thus, the element missing from Pomerantz – non-naturally occurring peptide linkers – is supplied by Krylov. The skilled worker would have had a reasonable expectation that Krylov's domains could be utilized to complex zinc fingers to which they are attached in view of Krylov's success in not only modifying their binding activity, but in making it stronger (i.e., more stable).

Krylov also teaches dimerization domains having the same sequence, meeting the limitations of clam 6. See e.g., id., page 2856, column 1, describing homo- and heterodimers, where the homodimers have "the same sequence."

Pomerantz describes dimers between ZFGD1 fusion protein, where each fusion contains the same zinc finger. Pomerantz, Abstract ("a dimeric zinc finger protein, ZFGD1"). This meets the requirements of claim 20.

In sum, we find that Pomerantz and Krylov disclose all elements of the subject matter recited in claims 5, 6, and 20. For the reasons discussed above, the skilled worker would have considered these claims obvious in view of Pomerantz's express suggestion to combine its teaching with Krylov (i.e., reference 19), and Krylov's disclosure that would have led the skilled worker to reasonably expect that the combination would work.

Summary

The rejections of claims 5, 6, and 20 are reversed. A new ground of a rejection has been entered under § 103 for claims 5, 6, and 20.

Time Period for Response

This decision contains a new ground of rejection pursuant to 37 CFR § 41.50(b) (effective September 13, 2004, 69 Fed. Reg. 49960 (August 12, 2004), 1286 Off. Gaz. Pat. Office 21 (September 7, 2004)). 37 CFR § 41.50(b) provides "[a] new ground of rejection pursuant to this paragraph shall not be considered final for judicial review."

37 CFR § 41.50(b) also provides that the appellant, WITHIN TWO MONTHS FROM THE DATE OF THE DECISION, must exercise one of the following two options with respect to the new ground of rejection to avoid termination of the appeal as to the rejected claims:

> (1) *Reopen prosecution.* Submit an appropriate amendment of the claims so rejected or new evidence relating to the claims so rejected, or both, and have the matter reconsidered by the examiner, in which event the proceeding will be remanded to the examiner. . . .
>
> (2) *Request rehearing.* Request that the proceeding be reheard under § 41.52 by the Board upon the same record. . . .

REVERSED, 37 CFR 41.50(b)

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